## Physico-chemical characterization of honey from Northern region of India

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#### **Abstract**

The physico-chemical characterization of honey samples collected from different locations varied in values of quality characteristics that ranged between  $3.85\pm0.16$  to  $5.63\pm0.01$  for pH,  $15.57\pm0.08$  to  $25.94\pm0.10$  for moisture,  $0.06\pm0.01$  to  $0.28\pm0.03$  for ash content,  $0.21\pm0.01$  to  $1.13\pm0.01$  for electrical conductivity,  $0.41\pm0.01$  to  $1.65\pm0.27$  for optical density,  $3.20\pm0.08$  to  $4.66\pm0.04$  for sucrose,  $34.65\pm0.09$  to  $42.06\pm0.24$  for acidity,  $66.95\pm0.62$  to  $70.79\pm0.55$  for total reducing sugars,  $28.71\pm0.50$  to  $31.45\pm0.42$  for glucose,  $36.88\pm0.61$   $42.26\pm0.10$  for fructose,  $1.19\pm0.03$  to  $1.47\pm0.02$  for F:G ratio,  $14.00\pm0.54$  to  $17.50\pm0.24$  for diastase activity and  $16.33\pm0.66$  to 20.90 for HMF content. All the samples as per tests performed were found of good quality and within the range set by FSSAI.

**Key words:** Honey, physicochemical characteristics, quality

Honey is the most important product of hive produced by bees from nectars, plant secretions and excretions of insects. Honey contains macro and micro nutrients, sugars (primarily fructose and glucose) and a variety of minor constituents such as phenolic compounds (Suarez et al. 2010; Minhas and Dhaliwal 2018). The composition of the honey is influenced by a number of biotic and abiotic factors such as floral sources, climatic conditions, soil quality, beekeeper operations, and regional variations (Thakur et al. 2021). Honey has many biological disease immunity boosting and further disease curing characteristics and is utilized in a wide variety of food products. Its sensory and physicochemical characterization and quality evaluation are crucial for the international trade as well as customer preferences (Belay et al. 2013).

The shelf life or quality of honey is influenced by HMF, sugars, acidity, diastase activity, and microbial growth in stored honey (Vijaykumar *et al.* 2020). Other significant elements affecting honey quality are the total amount of fructose, glucose, the fructose/glucose ratio, and the glucose/water ratio. The F: G ratio indicates the ability of honey to crystallize (Mhatre and Gude 2017). The amount of moisture in honey is an important indicator of fermentation and granulation. Low moisture content protects honey against microbial activity and thus can be stored for longer duration (Buba *et al.* 2013).

Various authorities, like the FSSAI standards for Honey, Codex Alimentarius, European Union, and International Honey Commission, among others, have established standard quality parameters that are used to evaluate the quality of honey (Parihar *et al.* 2020). The present study was undertaken to study various physical and chemical characteristics of honey collected from different locations of Himachal Pradesh, J&K and Punjab.

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#### **Materials and Methods**

#### **Sampling**

For the present study 60 samples of honey were collected from different locations of Himachal Pradesh and adjoining areas of J&K and Punjab states. The samples were labeled and stored in refrigerator at  $4\pm1$  °C.

#### рH

The method of AOAC (2012) was used to determine the pH of honey. Ten grams of honey was dissolved in 100 mL water and pH was measured directly by pH meter.

Table 1. Quality standards for honey given by FSSAI (2018)

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Parameters	Limits
pH	3.90 - 6.10
Moisture (%)	20.00
Ash content	Not more than 0.5% by
	mass
Electrical conductivity (mS/cm)	0.80
Sucrose (%)	5.00
F: Gratio	0.95-1.50
Acidity (meq/1000g)	50.00
HMF (mg/kg)	Not more than 80.00
Diastase activity (DN)	Not less than 3.00

## Moisture

Oven drying method (Ranganna, 2007) was used to check the moisture content in honey. Calculations were done using the formula:

Total moisture content (%) = 
$$\frac{Weight\ of\ fresh\ sample - Weight\ of\ dry\ sample}{Weight\ of\ fresh\ sample} \times 100$$

### Colour

Colour was determined by recording the optical density of honey at 560 nm in spectrophotometer (Townsend 1969).

#### Ash content

5 g of honey sample was weighed accurately and was placed in combustion pots. Then, the sample was incinerated at 550 °C in muffle furnace for 5 hours. The sample so reduced to ash was weighed after cooling at room temperature.

## **Electrical conductivity**

A solution of 20 g of honey was taken in 100 mL distilled water. The EC was measured by using a

digital electrical conductivity meter at 27 °C (Bogdanov *et al.* 2004).

#### Sucrose

Sucrose content was determined after inversion of honey solution. Fehling Solution A, Fehling Solution B and hydrochloric acid were used to find the percent sucrose content. Standard invert sugar solution was prepared by dissolving 0.95 g sucrose in 500 mL of water, followed by the addition of 2 mL of concentrated hydrochloric acid and thereafter neutralization with sodium carbonate. The calculations were done as per I.S.I. (1974).

Sucrose (% by weight) = [(Reducing sugars after inversion, per cent by weight) –

(Reducing sugars before inversion, per cent by weight)] x Sucrose factor (0.095).

#### F:G ratio

The glucose and fructose content was individually worked out as per I.S.I. (1974) and then the ratio was calculated using the formula:

$$F:G ratio = \frac{Fructose (\% by weight)}{Glucose (\% by weight)}$$

#### Acidity

Titrimetric method was employed to determine the free, lactonic and total acidity of honey (AOAC, 1984). Total acidity was calculated by adding free and lactonic acidities. The results were expressed in meq/kg of honey.

## **HMF** content

HMF content was calculated using the spectrophotometric method of White (1979). Calculations were done as per the formula:

$$HMF(mg/kg) = (A_{284}-A_{336}) \times 149.7 \times 5 \times D/W$$

Where,  $A_{284}$  = Absorbance at 284 nm;  $A_{336}$  = Absorbance at 336 nm; 149.7 = Constant; 5 = Theoretical nominal sample weight; D = Dilution, in case dilution is necessary; W = Weight in g of the honey sample

#### Diastase activity

The diastase activity was measured according to AOAC (2004). The diastase number was calculated according to the reference equation from the International Honey Commission.

Diastase number (DN) = (28.2 x absorbance)change at 620 nm after 10 minutes) + 2.64

### **Results and Discussion**

#### pН

The pH of honey ranged between 3.85±0.6 to 5.63±0.01 which was within the limits set by FSSAI (3.96-6.10). Similar lower range of pH value up to 3.81 was also documented by Kumar *et al.* (2018) of North Indian honey samples. The results of the study has close proximity to Anupama *et al.* (2003) who have documented pH in the range of 3.62 – 5.46 for Indian honey and Thakur *et al.* (2021) for pH value range of 4.65 to 5.94 for honey collected from different zones of Himachal Pradesh.

#### Moisture

During analysis of samples, highest moisture content was recorded in honey of Sullah (25.94%) which was at par with Una (22.61%), Nurpur (21.01%) and Baijnath (21.38%). The lowest moisture was recorded in the honey of Banihal (15.57%) and was statistically at par with honey samples of Nagrota Bagwan (19.49%), Kangra (19.08%), Jasur (20.14%), Hoshiarpur (20%) and Jammu (18.93%). The variation in the moisture content of honey might be due to different practices adopted by beekeepers, species of honey bees and storage conditions. In accordance with the present study, Anupama et al. (2003) and Akram et al. (2014) also have reported higher moisture content ranging between 17 to 22.6% and 22.87 to 26.70%, respectively. The results are also in line with the findings of Nanda et al. (2003) who documented moisture content in the range of 13.97 to 18.65% for North Indian honey samples.

## Ash content

Highest ash content was recorded in the honey of Baijnath (0.28 /5g) which was statistically at par with the honey of Nagrota Bagwan (0.15 g/5g), Kangra (0.17 g/5g), Sullah (0.18 g/5g), Una (0.24 g/5g) and Hoshiarpur (0.14 g/5g). The lowest ash content was recorded in the honey samples of Jasur (0.06 g/5g). Thi study has a close proximity to the records of Ahmed *et al.* (2014) who documented ash content in honey samples of their country ranging between 0.08 –0.39%.

## **Electrical conductivity**

Among samples, highest electrical conductivity of 1.13 mS/cm was recorded in the honey of Sullah followed by Baijnath (0.60) whereas lowest electrical conductivity was found in the honey of Banihal (0.21 mS/cm) followed by Jasur (0.27 mS/cm). The results resemble with the findings of Joshi *et al.* (1999), Qamer *et al.* (2008), Iftkhar *et al.* (2014) and Shobham *et al.* (2017) who reported similar values of electrical conductivity in honey.

#### Sucrose

The highest sucrose content of 4.66% was recorded in the honey sample from Jasur which was at par with the honey of Baijnath (3.98%), Nagrota Bagwan (4.07%), Kangra (3.92%), Sullah (4.55%), Nurpur (4.59%), Hoshiarpur (3.94%), Jammu (4.37%) and Banihal (3.84%). Lowest sucrose content was observed in the honey of Una (3.20%) which was statistically at par with honey from Baijnath (3.98%), Nagrota Bagwan (4.07%), Kangra (3.92%), Hoshiarpur (3.94%) and Banihal (3.84%). The results of the study get support by the findings of Kumar et al. (2018) who reported sucrose content within the permissible limit for North Indian honey and Kamboj et al. (2020) who also have documented sucrose content ranging from 3.11 to 3.59 in the honey of Punjab, Haryana, Himachal Pradesh and Rajasthan.

## F:G ratio

Highest F: G ratio of 1.47 was recorded for the honey of Banihal which was statistically at par with Una (1.33), Jasur (1.34), Nurpur (1.38), Baijnath (1.30) and Sullah (1.24). Lowest F: G ratio was recorded for the honey of Hoshiarpur (1.19). The F: G ratio of all the samples were within the acceptable range of FSSAI. The results are in agreement with the findings of Kamboj *et al.* (2020) who also reported F: G ratio ranging from 1.09-1.21 for the honey of Himachal Pradesh, Haryana, Rajasthan and Punjab. The study has also a support from the findings of Khandelwal *et al.* (2020).

Table 2. Physical and chemical characteristics of honey from different locations

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Location &	$^{\mathrm{Hd}}$	Moisture	Ash	Sucrose	Acidity	EC (mS/	F:G	Diastase	HMF	OD
No. of samples (N)		(%)	Content (g)	(%)	(meq/kg)	cm)	ratio	activity (DN)	content (mg/kg)	(560 nm)
Baijnath (n=5)	4.50±0.14	21.38±0.96	0.28±0.03	3.98±0.18	38.21±0.73	0.60±0.04 1.30±0.05	1.30±0.05	14.26±1.02	18.69±0.82	1.02±0.13
Nagrota (n=7)	$4.37\pm0.11$	$19.49\pm1.10$	$0.15\pm0.01$	$4.07\pm0.15$	$38.20\pm0.55$	$0.42\pm0.04$	$1.26\pm0.03$	$16.91\pm0.41$	20.90±1.09 1.22±0.21	$1.22\pm0.21$
Kangra (n=23)	$4.16\pm0.06$	$19.08\pm0.36$	$0.17\pm0.01$	$3.92\pm0.07$	39.13±0.36	$0.50\pm0.03$	$1.22\pm0.02$	$14.68\pm0.43$	19.92±0.36 0.95±0.11	$0.95\pm0.11$
Sullah(n=1)	$5.63\pm0.01$	$25.94\pm0.10$	$0.18\pm0.01$	$4.55\pm0.01$	$34.65\pm0.09$	$1.13\pm0.01$	$1.38\pm0.02$	$17.50\pm0.24$	$19.93\pm0.14$	$0.95\pm0.01$
Nurpur (n=6)	$4.18\pm0.06$	$21.01\pm0.62$	$0.10\pm0.01$	$4.59\pm0.05$	$38.72\pm0.46$	$0.46\pm0.04$	$1.24\pm0.04$	$14.00\pm0.54$	17.13±0.78	$1.01\pm0.09$
Jasur (n=5)	3.87±0.13	$20.14\pm0.57$	$0.06\pm0.01$	$4.66\pm0.04$	$40.34\pm0.62$	$0.27\pm0.01$ $1.34\pm0.07$	$1.34\pm0.07$	$15.18\pm0.15$	16.33±0.66 1.65±0.27	$1.65\pm0.27$
Una (n=3)	$4.41\pm0.08$	22.61±1.65	$0.24\pm0.03$	$3.20\pm0.08$	$38.14\pm0.42$	$0.59\pm0.03$	$1.33\pm0.02$	$14.99\pm1.01$	18.70±0.75	$0.65\pm0.01$
Hoshiarpur (n=5)	$3.95\pm0.04$	$20.00\pm0.62$	$0.14\pm0.02$	$3.94\pm0.17$	$39.02\pm0.59$	$0.41\pm0.02$	$1.19\pm0.03$	$14.02\pm0.82$	$20.58\pm0.52$	$1.33\pm0.14$
Jammu (n=4)	$3.85\pm0.16$	$18.93\pm0.89$	$0.10\pm0.04$	$4.37\pm0.17$	$41.34\pm0.44$	$0.32\pm0.03$ $1.20\pm0.04$	$1.20\pm0.04$	$17.09\pm0.76$	$20.15\pm0.87  0.49\pm0.05$	$0.49\pm0.05$
Banihal (n=1)	$3.97\pm0.1$	$15.57\pm0.08$	$0.13\pm0.01$	$3.84\pm0.01$	$42.06\pm0.24$	$0.21\pm0.01$	$1.47\pm0.02$	$15.66\pm0.16$	$19.62\pm0.26$	$0.42\pm0.01$
Mean	$4.29\pm0.04$	$20.37\pm0.26$	$0.16\pm0.00$	$4.11\pm0.04$	$38.98\pm0.20$	$0.49\pm0.01$	$1.25\pm0.01$	$15.43\pm0.23$	$19.20\pm0.25$	$1.03\pm0.06$
CD(P=0.05)	ı	5.42	0.42	0.91	4.17	1.17	0.23	4.88	5.18	1.24
SD	0.53	3.56	0.09	0.65	2.74	0.25	0.16	3.13	3.42	0.81
Results are expressed as mean + standard errors	las mean + stands	ard errors								

Results are expressed as mean  $\pm$  standard errors

### **Acidity**

Statistically, highest acidity was recorded for the honey of Banihal (42.06 meq/kg) which was at par with the honey of all the locations except Sullah (34.65 meq/kg). The lowest acidity was found in the honey of Sullah (34.65 meq/kg) which was statistically at par with Baijnath (38.21 meq/kg), Nagrota Bagwan (38.20 meq/kg), Nurpur (38.72 meq/kg) and Una (38.14 meq/kg). The present observations has support from the findings of Nanda *et al.* (2003) for acidity ranging between 23.67 to 43.00 meq/kg in the honey of Northern region of India and Akram *et al.* (2014) who documented acidity ranged from 24.32 to 37.55 meq/kg.

### **HMF** content

Highest HMF content was recorded in the honey of Nagrota Bagwan (20.90 mg/kg) followed by Sullah (19.93 mg/kg) whereas lowest HMF content was found in the honey of Jasur (16.33 mg/kg) followed by Nurpur (17.13 mg/kg). The study resembles with the findings of Yadav (1995) and Pasias *et al.* (2017) as they reported HMF content within the acceptable range.

### Diastase activity

The highest diastase value was recorded in the honey of Sullah (17.50 DN) followed by Jammu (17.09 DN) whereas lowest diastase activity was recorded for the honey of Nurpur (14.00 DN) followed by Hoshiarpur (14.02 DN). The results has support of the findings of Qamer *et al.* (2008), Nayik and Nanda *et al.* (2015), Vranic *et al.* (2017) who reported diastase activity in the range of 5.10 – 29.00 DN, 14.39 – 25.99 DN and 8.86 – 23.50 DN, respectively.

### Conclusion

The analysis of various physico-chemical parameters viz., pH, moisture, colour, ash content, electrical conductivity, sucrose content, F:G ratio, acidity, diastase activity and HMF content in the present study concluded that the honey from different locations of Himachal Pradesh, J&K and Punjab is of good quality as most of the analyzed parameters were in the range of approved limits by FSSAI.

**Conflict of interest:** The authors declare that there is no conflict of interest in this research paper.

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